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MEASUREMENT OF THE CATALYTIC POWER OF CATALASE

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I. INTRODUCTION

In this paper we propose to describe a new method and a new principle of determining the strength of catalase action. We do not endeavor to sum up all the literature existing, the excellent works of C. Oppenheimer[1] and H. Euler[2] having made this unnecessary. Of the literature which has appeared since 1909 we have taken only such work into account as showed an immediate connection with ours. Therefore we merely mention the elaborate work of G. B. Reed.[3]

The present problem originated from a proposed research on autofermentation in *Cannabis sativa* L. The first enzym to be dealt with was catalase. There were many difficulties to be overcome before we could start the work. The easiest way is to take commercial peroxide and let it act on crude plant juice, determining either the oxygen discharged by the method described by H. H. Bunzel[4] or the peroxide decomposed by means of titration with permanganate of potassium—the latter the method of nearly all other authors. With both methods we made determinations, but were very soon convinced that we were not ascertaining the actual strength of the enzym. One aim of the present paper, therefore, is to attempt to prove the inadequacy of the existing methods.

The value of a new method depends entirely on three factors: (1) The purity of the enzym; (2) the purity of the peroxide; and (3) the way of determining the action of the enzym on the peroxide. We took care only of the third factor in our experiments, though for the sake of completeness we mention all three. The first factor may vary according to the different aims of the research.

For studies in kinetics (R. O. Hertzog[5]), the enzym must be free from crystalloid, from peroxidase, and from impurities. For physiological research the methods of precipitation, be it with strong alcohol, inorganic salts or lead acetate, or by dialysis and centrifuging, enfeeble the enzym to

[1] Die Fermente, Zehnte Aufl. Leipzig, 1909.
[2] Grundlagen und Ergebnisse der Pflanzenchemie, 2ter Teil. 1909.
[3] Bot. Gaz. 1915–1918.
[4] Journ. Biol. Chem. 20. 1914.
[5] Zeitschr. Physik. Chem. 41. 1904; Oppenheimer, Part II.

[The Journal for June (7:223-260) was issued July 29, 1920.]

a marked degree and are therefore to be avoided as much as possible. All authors, however, agree that, although the influence of accompanying salts may not be very great, damage by acid is considerable (G. Senter[6]). Therefore the physiologist must take care to neutralize the juice. The neutralization with Na_2CO_3 worked in our experiments very satisfactorily. In experiments in which the same juice is used for several days, a few drops of toluol must be added as a preservative.

The impurity of commercial peroxide should not be overlooked. It contains acid (even the perhydrol of Merck) which must first be neutralized. Also, most of these peroxides contain a certain amount of acetanilide ($\pm 1/15$ percent). G. Senter has proved that aniline is poisonous to catalase. Therefore it is better to work with purified peroxide (methods by G. Bredig[7]). However, we shall try to show that, working with our method, the results are not greatly influenced by the impurities of the enzym or of the peroxide. We worked in these preliminary experiments with commercial 10-volume peroxide and freshly prepared, mostly undiluted, plant juice, neutralized during the grinding. We shall try the same method later on with purified chemicals and enzym.

The third factor, that of the method of determining the quantity of oxygen liberated or of peroxide decomposed, is the most important one. There are three methods for the quantitative measurement of catalase.

1. The method of Palladin (cited by A. Kasanski[8]) consists in measuring the height of the foam developed during the reaction. There is, however, no sufficient ratio between the intensity of the phenomenon and catalase activity. The purer the enzym, the smaller the volume of foam, etc.

2. Titration with potassium permanganate. This is the method used by nearly all authors, but we have considerable doubt that it will serve its purpose. In the first place, organic compounds of different kinds oxidize permanganate. Therefore the press juice of itself, has a certain oxidizing power. This power is difficult to measure, not only because the end point of the reaction (a permanent red color) is difficult to observe, but also because the fluid is often so much colored that color reactions cannot be measured. These facts are stated by P. Waentig and A. Steche[9] and by W. Issajew.[10] The latter does not mention his method, so that the value of his results could not be judged. Permanganate shows this uncertain end point also with all kinds of organic salts (citrates, malates, tartrates) as we were able to prove. Therefore it is not surprising that even excellent scientists like Bach sometimes made considerable errors with the titration method (A. M. Clover[11]). Notwithstanding our working with the usual

[6] Zeitschr. Physik. Chem. **51.** 1905.
[7] Zeitschr. Physik. Chem. **31.** 1899.
[8] Biochem. Zeitschr. **39.** 1912.
[9] Zeitschr. Physik. Chem. **72, 76, 79, 83.** 1911–1915.
[10] Zeitschr. Physik. Chem. **42.** 1905.
[11] Amer. Chem. Journ. **29.** 1904.

p[illegible]ution, we distrust the value of the numbers we obtained with the p[illegible]anganate method.

We come now to the manometrical methods. These lack the advan- [illegible] of the titration method in working under normal pressure. Further- [illegible] the fluid may reach the unfavorable condition of over-saturation w[illegible] gas (P. Waentig and A. Steche, *l.c.*). Of the authors whose work has b[illegible] done by this method we name: C. H. Appleman,[12] W. W. Bonns,[13] H[illegible]. Bunzel (*l.c.*), W. E. Burge,[14] C. Foa,[15] W. B. Magath,[16] W. Zaleski a[illegible] Anna Rosenberg.[17] The chief objections to this method are: (1) The pr[illegible]sure becomes higher during the reaction. We feel justified in disre- g[illegible]ing the effect of over-pressure as our check experiments have shown th[illegible] to be negligible. (2) The solution contains a great part of the oxygen. This is true only for narrow vessels, in which the surface is small in proportion to the volume of air. Even in the apparatus of H. H. Bunzel (*l.c.*) we feel that there is danger of the fluid becoming oversaturated. Bunzel tried to avoid this danger by shaking. But R. O. Hertzog (*l.c.*) cites a list of cases in which enzyms are destroyed by shaking. For instance, P. Waentig and A. Steche (*l.c.*) proved the destructive action of shaking on catalase.

It seemed clear to us, therefore, that if we chose the lesser of two evils, namely the manometrical method, we should take a container with a very broad bottom and a shallow layer of fluid. Experiments have shown us that the effect of shaking on the exchange of gas in such a column is minimal. In the short time of the catalase reaction the enzym is not injured by shaking. In more prolonged experiments (with oxidases for instance) it may be. There is another advantage in experimenting with a shallow layer of fluid since R. O. Hertzog (*l.c.*) proved that the catalase reaction is subject to the laws of diffusion, which is the most complete in thin layers.

II.

One more important criticism of nearly all methods of enzym determination is possible. To detect the fault we must start at the very beginning, at the definition of the word *enzym*. An enzym is a substance that *changes the velocity* of a reaction. Peroxide of hydrogen will decompose spontaneously but slowly. It will oxidize a certain amount in one month. Catalase changes the reaction time from one month to one minute. The only method theoretically justified would therefore be to determine the the time in which a reaction is completed under the influence of an enzym. That time is the measure of the enzym action.[18] If the reaction is monomo-

[12] Bot. Gaz. **50**. 1910.
[13] Ann. Mo. Bot. Garden **5**. 1918.
[14] Amer. Journ. Physiol. **44**. 1917.
[15] Biochem. Zeitschr. **11**. 1908.
[16] Journ. Biol. Chem. **24**. 1918.
[17] Biochem. Zeitschr. **33**. 1911.
[18] On the assumption that the reaction time *without* enzym is *very much* greater than that time *with* enzym.

lecular, and follows the law of mass action, the well-known formula of van't Hoff will apply:

$$\frac{dx}{dt} = k(a - x)$$

in which
- a = available amount,
- x = decomposed amount,
- t = time,
- k = reaction velocity.

Integration of this form $k = \frac{1}{0.4343t} \log \frac{a}{a - x}$ enables us to find the reaction velocity from a single determination. Concerning catalase a great variety of opinions exists in regard to the constancy of the reaction velocity. If the reaction velocity were proved to be practically constant, we should find, if x approaches its maximum value (let us say $\frac{999}{1000}a$):

$$k = \frac{3}{0.4343t}$$

or, k will be inversely proportional to t. In this case only would one be justified in measuring the so-called reaction-velocity, taking this as a comparative number for the "real" reaction-velocity, *i.e.*, $\frac{1}{\text{reaction time}}$. Excepting Bredig, who first called attention to this fact, F. A. F. C. Went[19] is the only author, so far as we know, who has tried to determine directly the time in which a reaction took place. He studied starch hydrolysis by the enzym of *Aspergillus niger*. His numbers are interpolated but still show marked properties.

In all cases, more or less scattered determinations (see, for example, figures in W. M. Bayliss[20] on glycerol-glucoside) must furnish the basis for the calculations. In our special case of catalase action, an *autographic method* which marks the time in which the reaction is ended offers a solution of this difficulty. Furthermore, this gives us opportunity to collect a far greater number of figures. As we learned after we had worked out the apparatus, the idea of an autographic record was not new.

C. Foa (*l.c.*) used a Mosso-plethysmograph and a revolving drum with soot paper to determine the action of different phenols on oxidase. He published his graphs without using them for calculation. A. Schultze (cited by Foa), used a self-recording manometer for measuring the CO_2 output in yeast activity. M. Antropoff studied autographically the periodical decomposition of peroxide by mercury. (Stephane Leduc[21] has explained his results in a peculiar way.)

[19] Verh. Kön. Akad. Wet. Amsterdam **27**. 1918.

[20] General physiology, 2nd edition. London, 1917.

[21] Théorie physico-chimique de la vie. Paris, 1910.

III.

We shall start now with the description of our own experiments.

We used a rather large reaction vessel connected with a manometer by a ground joint. The vessel was closed by a ground stopper. Joint and stopper were fastened on the bottle with strong rubber bands. On the ground stopper was sealed a small vessel with two holes. The small vessel contained the peroxide, while the larger held the enzym. The idea was borrowed from Haldane's well known apparatus for blood-gas determination. By turning the vessel in a plane perpendicular to the paper, the peroxide flowed from the smaller vessel into the larger one. In this plane the vessel could also be shaken. If the fluid contained catalase, oxygen would be liberated. This would increase the pressure in the vessel and the mercury column in the manometer would rise.

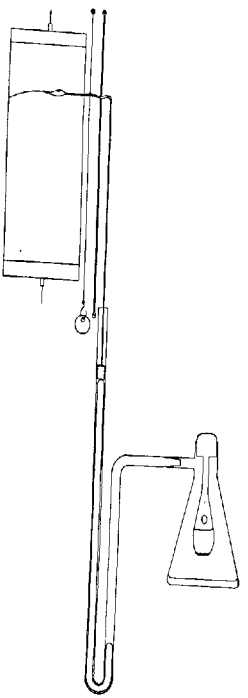

FIG. 1. Self-recording manometer with modified Haldane apparatus.

The autographic writer was simple to make. We had at least four methods from which to make our choice: (1) Transfer of the movement by levers; (2) transfer of the movement by air (Marey, Buisson); (3) direct record of mercury level by sensitive paper; (4) direct transfer.

The second method would not be the most efficient in our case, for, like the first method, it would record the results either enlarged or reduced. In the case of the third method we could not use coordinate paper. Therefore, we transcribed the pressure by means of a wooden float which carried a thin glass rod on which a glass pen was sealed at right angles to the rod. This we kept in position by a glass slide-bar and a weighted hair. This simple arrangement made it possible to register differences of $\frac{1}{50}$ mm. in the mercury level. The friction had an effect of $\frac{1}{10}$ mm. We therefore always calculated the records in millimeters. The efficient speed for the rotating drum was in our case one revolution in eight minutes for a diameter of 10 cm. The pen went over the distance of 1 mm. in $\frac{13}{8}$ seconds. The speed should be slower in the case of purer enzyms in which the action is feeble. The

flask stood inside the thermostat and could be shaken from the outside by means of a handle.

We obtained in normal cases a curve of the shape shown in figure 2.

At A (fig. 2) the peroxide is in contact with the enzym; at B the reaction begins; at C the reaction is completed. The distance A–B existed always

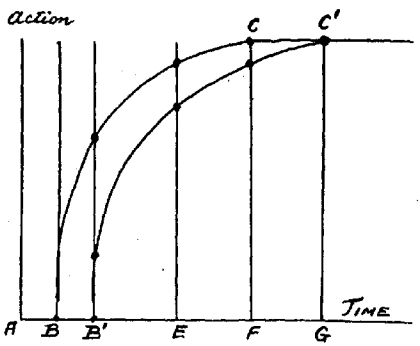

FIG. 2. For explanation see text.

and was not caused alone by the transportation time of the pressure. The distance A–B became greater when we used smaller quantities of enzym. This fact, namely, that oxygen is not immediately discharged, is mentioned only by P. Waentig and A. Steche (*l.c.*) With the use of the non-autographical methods, and especially with that of the titration method, this fact nearly always escapes observation. We saw the latency time (A–B) manifested, when the titration method was used, only in dilute enzym solutions. The small bubbles of oxygen were formed sometimes one minute after the beginning of the reaction. *It is clear, therefore, that the titration method not only is unfit to give us the end point of the reaction; it is unfit also to give us the moment of the beginning.*

The differing length of A–B with different enzym dilutions gives us a hint that the decomposition is caused by two successive reactions (A. Bach[22]), as follows:

1. $2H_2O_2 + 2A{=}O \rightarrow 2A\langle^{O-OH}_{OH}$ This unstable compound decomposes:
2. $2A\langle^{O-OH}_{OH} \rightarrow 2A\langle^{O}_{O} + 2H_2O$ This peroxide is decomposed:
3. $2A\langle^{O}_{O} \rightarrow 2A{=}O + O_2$ Summing up, we obtain
4. $2H_2O_2 \rightarrow 2H_2O + O_2$

[22] Chem. Ber. 36-42. 1904-1908.

This reaction (4) would be true when $A-B = 0$ seconds.

This is the reaction given by most authors. G. Bredig (*l.c.*), however, doubted the value of this equation. He found the reaction velocity constant. Therefore, he argues, the reaction must be monomolecular, and follow the scheme:

$$H_2O_2 \rightarrow H_2O + O$$

But the oxygen liberated is not atomic oxygen; it is not ionized, but molecular. T. H. Kastle and A. S. Loevenhart[23] defend on this ground the validity of the bimolecular equation. We are not able to follow their criticism of Bredig's work, and we will only remark that in the case in which $A-B>0$ the whole controversy seems to be solved. In fact, the first two reactions in the scheme of Bach are monomolecular.

IV.

We will compare now the action of two different quantities of enzym. The line A–B′–C′ (fig. 2) gives the action of the smaller quantity. What method must we follow to find out the ratio of their strengths?

1. *Reaction velocity.* The more accurate investigations deal with reaction velocity. We will show that the enzym is destroyed during the reaction (see below). Therefore the reaction velocity diminishes (sometimes very slightly) as nearly all authors have shown. (Issajew, *l.c.*, however, finds a constancy to the third decimal.) *The reaction velocity is therefore a misleading test for the strength of an enzym.*

2. *Amount of peroxide decomposed.* This method, though much used, has very little value, as figure 2 will demonstrate. A determination of the ratio in strength between A–B–C and A–B′–C′ would give:

At B′, 10:5;
E, 10:7;
F, 10:9;
G, 10:10.

Still we find in the literature on the subject, expressions like this: "There is three times as much catalase in the body wall of *Ascaris suum* as in the leg muscles of *Rana pipiens*" (Magath, *l.c.*).

3. A better method is the direct measurement of the reaction time (see above, II). This is possible only with a self-recording apparatus.

We prepared our materials by the method thus described. The tops of female hemp plants were ground in a meat-grinder with a small amount of powdered Na_2CO_3. The ground substance was then squeezed in a fruit press. The turbid fluid obtained is very stable and still strongly active after the lapse of fourteen days. The determinations all took place at 20° C. The peroxide was the usual commercial 10-volume H_2O_2, which

[23] Amer. Chem. Journ. 29. 1903.

contains acetanilide enough to damage the enzym during the reaction (see below). We expect to repeat the experiments under standard conditions and ask the reader therefore to consider this paper as a preliminary account.

The time in which the reaction on 2 cc. peroxide was completed was in one case:

For 4 cc. extract, 15 mm. (15 $\times \frac{1}{8}^3$ sec.);
for 3 cc. extract, 21 mm. (21 $\times \frac{1}{8}^3$ sec.);
for 2 cc. extract, 29.5 mm. (29.5 $\times \frac{1}{8}^3$ sec.);
for 1 cc. extract, 59 mm. (59 $\times \frac{1}{8}^3$ sec.);
for $\frac{1}{2}$ cc. extract, 118 mm. (118 $\times \frac{1}{8}^3$ sec.).

We can state that the reaction time is inversely proportional to the amount of enzym, E (amount of enzym) $\times$ T (reaction time in units of $\frac{1}{8}^3$ seconds) thus being constant.

Again:

For 4 cc. extract, $E \times T = 60$;
for 3 cc. extract, $E \times T = 63$;
for 2 cc. extract, $E \times T = 59$;
for 1 cc. extract, $E \times T = 59$;
for $\frac{1}{2}$ cc. extract, $E \times T = 59$.

In another case we obtained these results:

For 4 cc. extract, $E \times T = 24$;
for 3 cc. extract, $E \times T = 27$;
for 2 cc. extract, $E \times T = 26$;
for 1 cc. extract, $E \times T = 25$;
for $\frac{1}{2}$ cc. extract, $E \times T = 24.5$.

These numbers strikingly show the value of the autographical method.

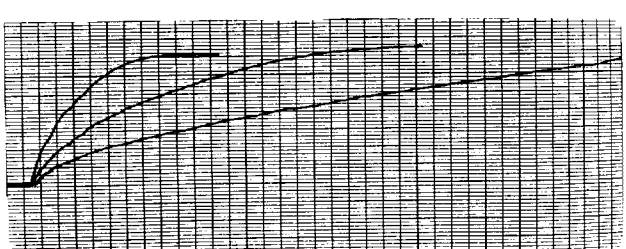

FIG. 3. Autogram. Effects of different quantities of enzym.

Figure 3 gives still another proof for 3 curves, taken with 4, 2, and 1 cc. of extract respectively, and 4 cc. 10-vol. H_2O_2. Different amounts of

peroxide change the time in the same manner. Figure 4 will illustrate this.

cc. of extract react with:

	Time in "mm."	T peroxide
4 cc. 10-vol. peroxide	45	11.3 (45) 4
3 cc. 10-vol. peroxide	34	11.3 (34) 3
2 cc. 10-vol. peroxide	22	11. (22) 2
1 cc. 10-vol. peroxide	10	10 (10) 1

The times are proportional to the amount of peroxide.

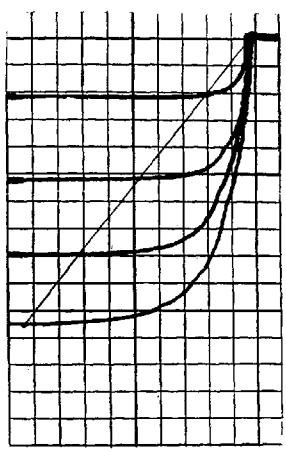

FIG. 4. Autogram. Effects of different quantities of peroxide.

To compare these results with the reaction velocities, we used the following method: We imagined the assymptote extended to the ordinate axis. Then the quantity $(a - x)$ of the formula

$$\frac{dx}{dt} = c\,(a - x)$$

will be the distance from a given point of the curve to the assymptote. We call it D. We can write for a point of the curve P:

$$tg\alpha_P = K_P D_P, \text{ or } K_P = \frac{tg\alpha_P}{D_P},$$

α_P being the angle between the tangent at the point P and the time axis.

We had only to measure α_P and D_P and we could read immedi[illegible]y on the slide rule the resulting K_P. We averaged a great number of K[illegible]aken from different curves. We also calculated the probable error. It pr[illegible]ed to affect the units only. This must be taken into account.

Extract in cc.	Peroxide in cc.	Number of Experiments	Reaction Velocity	Peroxi[illegible] Enz[illegible]
$\frac{1}{2}$	2	4	41×10^{-4}	$164 \times$ [illegible]
1	2	7	110×10^{-4}	$220 \times$ [illegible]
2	2	11	257×10^{-4}	$257 \times$ [illegible]
2	4	4	109×10^{-4}	$218 \times$ [illegible]
3	2	4	396×10^{-4}	$264 \times$ [illegible]
4	2	1	535×10^{-4}	263×10^{-4}
4	4	4	261×10^{-4}	261×10^{-4}
1	4	4	47×10^{-4}	188×10^{-4}

Considering the irregularity of the curves due to the poor clockwork and the possible differences in strength of the enzym solutions, $\frac{PK}{E}$ is a fairly approximately constant number. But it would take 39 determinations of this sort to prove what one determination of reaction time gave us, namely, that the reaction follows the law of mass action.

We tried to compare the curves obtained with mathematically constructed logarithmic lines. (Kapteyn used a similar method for Gaussian curves). We constructed several lines

$$t = c \log \frac{a}{a - x}$$

for c varying from 0.1 to 2 and a = 2 cm. We found in one case:

Calculated from Curve	Amount extract	C × E
0.15	4 cc.	0.6
0.2	3 cc.	0.6
0.3	2 cc.	0.6
0.6	1 cc.	0.6
1.2	0.5 cc.	0.6

Perhaps this method will be found to be the most practical and accurate.

The line A–B in which, according to our idea, the first part of the reaction must take place, becomes long enough to be measured in very feeble enzym concentrations only. Figure 5 shows curves run with 3 cc. peroxide and 4, 3, 2, 1, 0.5, 0.2, 0.1, and 0.05 cc. extract respectively, all diluted to 4 cc. fluid. The reaction started at the thick vertical line. The latency time caused by the apparatus (fig. 5) seems to be $2 \times \frac{13}{8}$ seconds. So we had to subtract 2 from the length A–B.

Latency time × *amount of enzym* seems to be more or less constant. But to draw conclusions from these facts seems premature.

extract	A–B	A–B Calculated for E × (A–B) = 1.5	E × (A–B)
4	Not measurable	0.38	—
3		0.5	—
2		0.75	—
1		1.5	—
0.5	1*	3	—
0.2	7	7.5	1.4
0.1	15	15	1.5
0.05	32	30	1.6

* Out of place.

V.

There is still one assumption which we have not proved. How do we know that the reaction is finished at the point C of our curve (fig. 2), where

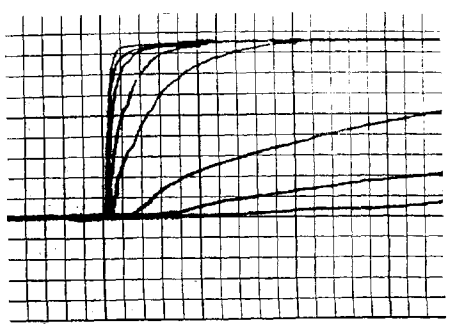

FIG. 5. Autogram showing latency times.

the curve becomes assymptote to the time axis? To determine this we have only to calibrate our flask. If the volume of the container to the level of the mercury is V_1, the volume of the peroxide V_H, and that of the extract V_E, the remaining volume before the beginning of the reaction will be $V_1 - (V_H - V_E)$. After the liberation of oxygen the mercury is forced down a cm., the volume of 1 cm. to be V_c cc., so the volume after the reaction will be

$$V_1 = (V_H + V_E) + aV_c.$$

If the temperature is constant during the experiment, we can use the simple formula of Boyle. If the pressure before the experiment be H cm. mercury, it will be $(H + 2a)$ after the reaction. We get for the volume after the

reaction:

$$V_2 = \left\{ \frac{V_1 - (V_H + V_E) + aV_c}{H} \right\} (H + 2a)$$

or the oxygen produced

$$V_2 - \{V_1 - (V_H + V_E)\} = \frac{a}{H}\left[2\{V_1 - (V_H + V_E)\} + V_c(H + 2a)\right].$$

In one case we found:

$V_1 = 272.19$
$V_H = 2$
$V_E = 2$
$a = 2.3$
$H = 76$
$V_c = 0.29$

Oxygen discharged:

$$\frac{2.3}{76}\left[2 \times 268.19 + 0.29 \times 80.6\right] = 17.2 \text{ cc.}$$

Now the original peroxide was supposed to be 10-volume. We checked the experiment by titration at the same temperature and found 8.7-volume. This signifies that 2 cc. peroxide would yield 17.4 cc. oxygen.

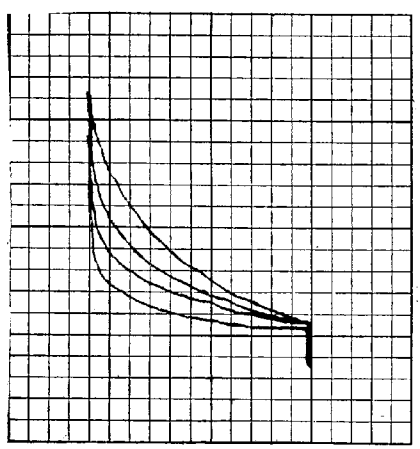

FIG. 6. Autogram showing the influence of successive doses of peroxide.

Thus we see that the autographic method can be used to determine the strength of a peroxide solution.

We can make this clear with an experiment done with 7.5-volume peroxide and 4 cc. extract. The autographic record showed:

Peroxide in cc.	Final Pressure in cm.	cc. Oxygen Calculated From Titration	cc. Oxygen Calculated From Formula
1	1.2	7.5	8.2
2	2.2	15.	16.2
3	3.3	22.5	23.0
4	4.2	30.	28.2
5	5.6	37.5	37.3
6	6.8	45.	45.
7	8.	52.5	52.5
8	9.1	60.	59.6

Successive doses of 4 cc. peroxide on the same 4 cc. extract had the results shown in figure 6. There are two explanations possible. Either (1) the dilution of the solution affects the strength of the enzym, or (2) the enzym is "poisoned" by the peroxide.

We now made a determination of the influence of dilution by means of the titration method. From the results of this experiment we may conclude that the action of catalase does not vary with its dilution or with the quantity of the peroxide, but only with the absolute quantity of the enzym itself.

The results were:

Constitution of the Enzym Solution	Peroxide	Percent Peroxide Decomposed after 1 Minute
1 cc. extract, 0 H_2O	2 cc.	34.6%
1 cc. extract, 3 H_2O	2 cc.	29.8%
1 cc. extract, 8 H_2O	2 cc.	33.3%
1 cc. extract, 15 H_2O	2 cc.	30.9%
1 cc. extract, 24 H_2O	3 cc.	30.3%
1 cc. extract, 35 H_2O	2 cc.	31.8%
1 cc. extract, 48 H_2O	2 cc.	32.9%
1 cc. extract, 63 H_2O	4 cc.	26.9%

So only the second assumption is valid, the enzym is destroyed by the peroxide.

The reaction times of the successive amounts were:

	Time	Strength = $\frac{1}{\text{time}}$
1st dose	52 (× 1 5/8 sec.)	100
2d dose	58	89
3d dose	65	80
4th dose	75	69

± 10 percent of the enzym is destroyed during every successive reaction. This decrease in the reaction velocity supports the unproved assumptions of Bredig (*l.c.*).

The influence of alkali is very marked. Enzym solutions neutralized with Na_2CO_3 hold their power for days. Even neutralized hemp powder that had been dried for two weeks showed marked activity. There is a strong possibility that the alkali works as a "peptisator" on the enzym. Many peptisators are known in colloid chemistry, alkali acting very strongly

on albuminoids (Graham). The protein character of catalase is i[illegible]bable (Waentig and Steche, *l.c.*). The assumption of an α and a β [illegible]lase, proposed originally by O. Loew[24] would in that case be superfluous ([illegible]pare E. Pozzi-Escot[25]) and Appleman (*l.c.*). The activity of the catalase [illegible]clines very slowly on filtering, especially if the solution has been previously [illegible]tral-ized. In the latter case the activity decreased only 8 percent.

Unneutralized juices lose their catalytic power very soon. W[illegible]und, for instance, in one case:

	Standing	Reaction Time	Succ[illegible]
2 cc. extract neutralized	5′	46 units	100
2 cc. extract unneutralized	5′	85 units	84
2 cc. extract unneutralized	10′	151 units	34
2 cc. extract unneutralized	120′		0

There is evidence that this reaction follows also a logarithmic line.

Attempts to prepare the enzym in pure condition have failed. Unlike peroxidase, catalase adheres with a great tenacity to the alcohol precipitate.

We have refrained in the foregoing from discussing the physiological questions suggested by or even suggesting our work, for such a research can start only after the methods are worked out satisfactorily.

Summary

1. A review is given of the literature concerning the question. Difficulties and inaccuracies in several methods are pointed out.

2. According to the definition of an enzym, the reaction time is the only valid index of its strength. This strength can best be measured by an autographical method.

3. An autographical method is given. The method shows the evidence of two successive reactions.

4. The enzym is more or less injured or destroyed during the reaction. In most reactions the time is too short to influence markedly the logarithmic curve.

5. The method given is adapted to determine the strength of a peroxide solution.

6. There is evidence that the two different catalases are different degrees of peptisation of the same substance.

We wish to express our grateful appreciation of the encouragement which Professor G. J. Peirce, by his criticism and suggestions, has given us. We also are indebted to Professors L. L. Burlingame and S. W. Young for valuable advice.

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[24] U. S. Dept. Agric. Report 68. 1901.

[25] Amer. Chem. Journ. 29. 1903.

EARLY STAGES IN THE DEVELOPMENT OF CERTAIN PACHYPSYLLA GALLS ON CELTIS

B. W. WELLS

One of the most interesting angles from which the insect gall problem may be attacked is that involving the early stages of gall formation. The present study is a morphological one dealing with the beginning stages of two Pachypsylla (Fam. Psyllidae of the Hemiptera) galls as they develop on the leaves of the hackberry (Celtis).

THE GALLS

The galls concerned are common ones in their respective regions.

Pachypsylla mamma Riley is found on *Celtis occidentalis* L. It is a hemispheric to subcylindric outgrowth projecting from the under side of the hackberry leaf (Pl. XVIII, fig. 2). Above, on the opposite side, is a prominent depression, in the center of which is a minute conical process. Interiorly there is an inverted dome-shaped larval cavity lined by soft nutritive tissue; this latter in turn is invested by masses of hard, "protective" sclerenchyma. For a detailed discussion of the adult gall, the reader is referred to an earlier paper of the author's (8).

Pachypsylla asteriscus Riley occurs on *Celtis mississippiensis* Bosc. (fig. 1, *c*, and fig. 7). It projects from both sides of the leaf, the part on the under side assuming the shape of a much abbreviated Convolvulus corolla, the part above consisting of a slender, straight process. These processes are attached to a blister-like enlargement in the plane of the leaf, in the interior of which is the lens-shaped larval chamber. Well defined layers of sclerenchyma occur in this gall, bounding the nutritive tissue.

Both of these galls are prosoplasmas or "higher" galls characterized by definite constitution and growth period and possessing highly specific forms and differentiation structures, which latter unusual characters are induced to appear under the action of the specific stimulus developed by the insect larva.

EARLIER WORK

No ontogenetical studies of the beginning stages of the galls formed by this genus of insects have heretofore been made. A few studies, however, have been made on the galls of other genera of the Hemiptera which may properly be presented here.

Prillieux (6) finds, in his study of the woolly apple aphis (*Schizoneura lanigera* Hausm.) gall on the apple twig, the following facts: No change

takes place in the cortical cells pierced by the proboscis of the insect other than the reaction to the mouth parts in them by laying down an "organic sheath" around these mouth parts. The living cells of the wood beneath the mouth parts proliferate strongly, building up a mass of hyperplastic parenchyma in which remnants of ducts are scattered. Multinucleate cells are reported present, but the author does not state in what cells this condition occurs.

Petri (4), in a comprehensive study of the grape Phylloxera root gall, finds the following phenomena to be exhibited: A "warty deposit" of calcium pectate is formed around the setae of the insect after their insertion, by the cytoplasm of the cells in which the setae are present. On the exterior of this sheath Petri believed he demonstrated a layer of tannic substance which upon oxidation gave the characteristic brown color to the old sheaths. The first important changes in the surrounding tissues are the sudden cessation of growth and the non-appearance of differentiation in all near-by cells. These cells show hypertrophy of their nuclei. A short distance from the mouth parts a "ringwall" of hyperplastic tissue springs up.

Rosen (7) studied the grape Phylloxera leaf gall with the following results: The initial depression is produced through "a lessened growth of the attacked mesophyll." "After three to four days of insect attack, the lower half of the leaf tissue which surrounds the portion in which the proboscis is inserted has proliferated enormously. The whole thickness of the leaf in the region immediately around the proboscis shows no proliferation. That portion of the leaf which is beneath the insect does not proliferate but the upper half at the sides of the insect grows upwards and forms the walls of a large insect cavity. Upper epidermal cells and several layers of mesophyll cells in the portion of the gall below the insect, show peculiar thickening and dissolution of their walls." "The investigation establishes the fact that the proboscis may pass through the entire thickness of the leaf." "The continuous sucking action by the insect at one fixed point for fifteen days is believed to be the initial stimulus for gall development."

METHODS

The material of *Pachypsylla mamma* and observational data concerning it were obtained in and near Manhattan, Kansas. That of *P. asterisca* was acquired in northeastern Texas.

At the critical time in the spring, hatching of the nymphs and gall initiation were observed in the field and laboratory, making possible the fixation of gall material in its earliest stages. All material was fixed in weak chrom-acetic killing fluid (Schaffner's formula), embedded in paraffin, cut 10 microns thick and stained with Flemming's triple stain. All histological drawings were made with the aid of a camera lucida.

Initial Stages of the Galls

Before attacking the problem of primary cecidium ontogenesis, a very brief statement of the life history of the insect will be given leading up to gall initiation. The following deals with *P. mamma*. *P. asteriscus* has an almost identical life history.

The adults, which are formed immediately after the escape from the galls of the fully grown nymphs in the fall of the year, overwinter in the bark crevices or in ground debris. After mating in early spring, the females lay their eggs on the under side of the young leaves as they begin to protrude from the buds. The point at which the egg is attached is commonly killed, this killing resulting in a prominent hole in the mature leaf. This explains, in part, the presence of numerous perforations in gall-infested leaves. The minute oval eggs (0.3 mm. long) hatch in 2–3 days, the nymph immediately migrating to the upper side of the very young leaf, where, after reaching a position near a principal vein, it settles down to initiate gall development. Once the growing gall has engulfed it, it is a prisoner until its escape the following fall as a mature nymph.

The nymph at the time of gall initiation is a minute, salmon-colored, flattened insect, oval in outline, and measures 0.22 mm. in length. On the ventral side, the setae, which in this minute insect are perfectly formed, extend from the body at a place slightly anterior to the median point. The setal puncturing mechanism measures but little over 1 micron in transverse diameter. There are no special structural modifications in these gall-making nymphs which distinguish them from the large numbers of non-gall-forming Hemipterous larvae.

Under the binocular microscope, the early superficial conditions in gall development may be easily observed and are as follows: The insect's body is pressed close to the upper side of the leaf; a shallow downward evagination forms, lowering the insect into the body of the leaf; when the upper side of the insect has been lowered to the level of the leaf surface, a very rapid upward growth of the leaf tissue surrounding the insect takes place, appearing first like a crater but finally as a closed cone completely covering the nymph. Both *P. mamma* and *P. asteriscus* galls are characterized in their initial stages by this combination of the diverticulum and walled conditions ("Umwallungen" of Küster). The *P. mamma* gall in its further development emphasizes the diverticulum character, the original "cover-cone" developing but slightly, while in the case of *P. asteriscus* no prominent evagination occurs but the cover-cone grows into the prominent, slender, subcylindric process so characteristic of this gall.

Histological Phenomena

At the time when the nymph inserts its setae into the embryo leaf, the leaf cells are not in the primordial condition of undifferentiation but show

distinct though partial differentiation; the upper epidermis is very well defined, being composed of large cuboidal cells (fig. 3, at right); the mesophyll shows three distinct layers, and the lower epidermis is set off from the other layers by the minute size of the units composing it. The chloroplasts of the mesophyll cells are well developed as to size and number.

A number of modifications occur coincidently following the insertion of the setal proboscis. These are: (1) The reaction of the cytoplasm of the cells penetrated by the setal structure to this foreign structure, by laying down around it a deposit of organic substance in the form of a very thin, uniform membrane. This structure thus constitutes a definite sheath. (2) A marked hypertrophy of the lower epidermal cells and in a lesser degree of the adjacent mesophyll cells. (3) Hyperplasia sets in in the middle region of the mesophyll surrounding the end of the proboscis. This, however, is greatly restricted. In the case of the *P. mamma* gall (fig. 3), wall formation accompanies the nuclear divisions almost invariably in the early stages; while in the case of *P. asteriscus* wall construction does not occur, the original cells of the mesophyll tiers thus being left almost undisturbed, the cells, however, containing many nuclei. This is best demonstrated in a somewhat later stage (fig. 5*b*). (4) Partial degeneration of the chloroplasts of cells beneath the insect, involving loss of chlorophyll and more or less reduction in size. (5) An increase in the size of the nuclei as compared with those of the normal parts.

The total result of the early hypertrophic and hyperplastic changes is the formation of a saucer-shaped depression in which the nymph (not shown in section) passively lies (fig. 3). This depression or evagination is produced chiefly through the hypertrophy of the elements on the under side of the leaf or that opposite to the insect.

Figure 4 presents a median section of a later stage of the *P. mamma* gall. It will be noted that, compared to the tissue lying at some distance from the larva, the tissue immediately beneath the insect and adjacent to the proboscis shows a marked inhibition of cell-divisional activity. This condition is very characteristic of both galls. These non-dividing cells, however, show the same high protoplasmic content that the actively dividing cells do, so that, when the section is viewed as a whole, there appears to be a zone of meristematic tissue traversing the young gall (shaded cells, fig. 4).

The whole situation, despite the fact of the local growth inhibition mentioned, when contrasted with the condition in a normal partially differentiated leaf, shows that in the early stages of cecidogenesis a process of dedifferentiation is going on, throwing the tissue back into a homogeneous condition. Were events to stop here (fig. 4), we should have a typical kataplasma showing the retrogressive changes of that type of overgrowth. The gall in its further development, however, differentiates into a highly specific prosoplasma, thus furnishing a characteristic example of gall ontogeny recapitulating gall phylogeny.

A still older stage (1 mm. long diameter) of a *P. mamma* gall (median section) is shown in figure 9. The cover-cone has attained almost its maximum size (in the adult gall it is a minute papilla), and the characteristic subhemispheric form of the gall has definitely appeared. The occurrence on the very young galls of the large trichomes is worthy of note. These are highly evanescent, never being seen on the adult structure. A presentation of the histology of the nearly mature gall is given in an earlier paper of the writer's (8).

The early histogenesis of a *P. asteriscus* gall is different from that of the *P. mamma* gall. The differences are of course related to the specificity of the galls.

Figure 5 shows the outline of a median section of a very young *P. asteriscus* gall (1 mm. long diameter). At this early stage the fundamental form of the mature gall (fig. 7) is appearing in the prominent cover-cone, the circular outgrowth beneath which in section is exhibited as two lateral processes. The thickness of the region beneath the insect is definitely less than that of the normal leaf as seen to left and right, indicating a marked inhibition of growth in those tissues.

In figure 5*a* are presented the tissue conditions of a critical portion of the section shown in figure 5. Here, as in the case of the *P. mamma* gall, a meristem-like layer is well defined. The details of these two layers and other cellular features are shown in figure 5*b*, which presents the region immediately surrounding the mouth parts. This region, it will be noted, with the exception of a small portion of it directly beneath the sheath-enclosed mouth parts, has maintained the original five-layered condition of the young leaf. Though slightly excessive nuclear division has taken place in the zone of growth inhibition, wall formation has not occurred, the result being the appearance of multinucleate or "giant" cells, the largest of which is that one in which the end of the proboscis terminates. The presence of such a giant cell as the one last mentioned, is also noted in the *P. mamma* galls (fig. 6). Further discussion of this giant cell situation will be given later.

Rosen (7) found an inhibition of growth in the floor of the grape Phylloxera leaf gall but reports no multinucleate condition.

Differing in still another character from the *P. mamma* gall, that produced by *P. asteriscus* shows the very early differentiation of a sclerenchyma layer through the deposition on the walls of the lower hypodermal cells of bands of lignin, simulating the scalariform type of lignification (fig. 5*b*).

The curious condition of the cell in the upper right-hand corner of the section (fig. 5*b*), the cell being almost empty and a rivet-shaped plug of deeply staining matter inserted in the wall, is undoubtedly due to mechanical puncture by one of the two stout bristles with which each leg of the nymph is armed. More injury of this character does not occur probably because of the extreme passivity which characterizes the insect throughout the period of gall formation.

The Sheath

The results of this study are in accordance with Prillieux's [illegible] and Petri's (4) observations that an organic membrane is deposited by the cytoplasm around the setal proboscis (figs. 5*b*, 6, 8, 10). I found as did Petri in the grape Phylloxera root gall that this membrane is composed of calcium pectate, but was unable as he was to demonstrate a layer of tannic substance on its exterior. In Petri's material the deposit was irregularly laid down or, as he described it, appeared "warty," while in mine it was deposited with remarkable uniformity as to thickness. This sheath is open at its end, thus making possible the direct movement of cellular substances into the end of the setal proboscis. This opening can be demonstrated only with the oil immersion objective in favorable sections (fig. 5*b*), for in many instances the end of the tubular sheath is surrounded by a dense mass of cytoplasmic granules. The nature of this mass I was unable to determine.

Figure 8 illustrates a case in which the insect partially withdrew the setae, then plunged them in again in a different direction. Figure 10 shows an extremely rare condition in which the nymph has withdrawn the mouth parts completely and twice reinserted them. The setae were found as shown, broken off and sticking in the shortest of the three sheaths. The middle sheath is distinctly abnormal. This section shows a condition commonly observed, *viz.*, the projection of the sheath structure beyond the surface of the outer cells.

Examination of half-grown galls and of older ones has shown but one sheath present, indicating that but one insertion of the proboscis takes place, the insect remaining passive and maintaining a stable position for relatively long periods of time. Rosen (7) found the same to be true in the grape Phylloxera leaf gall. Rosen's figure 4 shows a perfect sheath which he inadvertently misinterpreted as "proboscis."

Since in all studies to date of Hemipterous insect cecidiogenesis, an organic sheath has been found surrounding the setal mouth parts, we have reason to believe that the phenomenon is a general one for that group of zoocecidia.

Multinucleate Cells

Multinucleate or giant cells are not unknown in gall tissues, and even are not absent from normal tissues according to Beer and Arber (1), who have found the phenomenon in the stems of 50 dicots and 17 monocots. Prankerd's (5) studies of the occurrence of multinucleate cells in normal tissue are of interest in this connection. He believes they are mostly formed by amitosis. He finds in many instances that a wall is formed later by the two protoplasts resulting from a single amitotic division. Küster (3) presents a number of records of cecidial giant cells in many of which amitosis is reported as the mode of origin of the nuclei. In one instance, the Erineum

mite gall on the leaf of the European linden, Küster found, in addition, the degeneration of one nucleus to be a constant phenomenon.

The multinucleate cells found in this study are of much interest, not only because such cells have been rarely reported in the higher galls, but also because a special situation was found in which many of the nuclei were undergoing a process of disintegration.

In the *P. asteriscus* gall, by the time the gall has attained a length of 1 mm. many of the cells, both the one in which the stylets end and certain of those in the immediate neighborhood, show more than one nucleus (fig. 5*b*). The median cell holding the opening of the sheath-enclosed proboscis has generally the largest number, as many as four to six being visible in one focal plane. The other cells possess fewer, more than two being seldom found definitely showing in the same focal plane. Only a few cells in such a section as shown in figure 5*b* are thus seen (oil immersion lens), but these are unmistakably multinucleate.

The nuclei within a particular cell upon critical examination do not appear to be all in the same condition. One commonly, or at the most two of them, may be regarded as normal, while the others exhibit varying stages of disintegration (fig. 5*c*, scale same as fig. 9*a*). In this disintegration process the nuclear membrane disappears, and a prominent vacuole develops in place of a part or all of the nuclear body. The nucleolus persists quite unchanged and may often be found stranded in the cytoplasm near a vacuole.

In the 1 mm. gall of *P. mamma* practically no multinucleate cells are found aside from the large central one in which the mouth parts terminate. But in much older material (the half-grown gall) excellent examples of the giant-cell condition are found. In these, as in the cells of the *P. asteriscus* gall, all stages of disintegration of the nuclei excepting one or two are plainly evident (fig. 9*a*). The problem of the mode of origin of these nuclei is an important one since in some instances, such as the giant cells of the nematode (Heterodera) galls, nuclear proliferation has been reported as amitotic in character. After a most painstaking examination of my slides I am strongly inclined to interpret the situation in terms of amitosis. I am not able, however, to furnish direct positive proof.

The total absence of mitotic figures from the cells of the region concerned might be due merely to the fact that this region is characterized by a marked inhibition of growth as compared to adjacent regions. Individual cells having their origin in the normal embryo leaf before the insect's attack, later, under the inhibiting influence exercised on the three cell layers beneath the nymph, retain their integrity even into the adult gall stage, changing only in the matter of numerical nuclear increase.

This latter change is then followed by degeneration of certain of the nuclei. Since the number of nuclei per cell is always relatively low, seldom if ever going over eight, and since the divisions producing these are scattered

over a relatively long period of time (one to four weeks roughly), the chances of catching a nucleus in the act of division are very remote. This of course is true whether the division is of the mitotic or of the amitotic type. For this reason if for no other it is impossible to determine definitely the nature of the nuclear cleavage.

The evidence for amitosis is of an indirect character, such as the frequent occurrence of lobulate nuclei; the very rare display of a constricted nucleus; the frequent occurrence of two nuclei in contiguity; and the very irregular distribution of the nuclei in the cell. But, as heretofore stated, the extremely high infrequency of divisions of any kind makes it quite impossible to determine the nature of the divisions definitely.

Differential Growth between Larva and Gall

Data should be given concerning the difference in time of occurrence of the grand periods of growth of the larva and gall respectively, as this is a matter of more than passing interest. This difference is readily brought out by the following table:

Measurements of larva and gall at different stages of their concomitant development

	Width of larva	Height of gall
Stage 1	0.20 mm.	0.14 mm.
Stage 2	0.22 mm.	0.80 mm.
Stage 3	0.31 mm.	3.55 mm.
Stage 4	0.40 mm.	3.75 mm.
Stage 5	1.69 mm.	5.00 mm.

The grand period of growth for the gall is that of its early existence, while that of the larva comes later when the gall is more than half grown. This inhibition of growth in the larva for an extended period, which is coincident with gall morphogenesis, is in the mind of the writer a very significant phenomenon. Just what it implies in its entirety is quite unknown, but we may feel reasonably sure that it is related in some manner to the production of the gall-making stimulus. The energy of the larva appears at first to be consumed in producing the stimulus, and only when gall development is well on its way to maturity is this energy released for the anabolic processes of the larva itself. This is of course but an assumed generalization which cannot be proved until the problem of the nature of the stimulus involved has been cleared up.

The Stimulus Problem

In many instances the discovery of special structures has been of much value in interpreting function. When we raise the physiological problems of the nature of the gall-forming stimulus in these prosoplasmas and look for some hints concerning it from structural conditions, it must be confessed that none are given. I cannot find a single structural fact produced by

any one in the study of the initial stages of Hemiptera galls, that throws any light whatever upon the profound problem of the nature of the highly specific stimulus applied by the insect to the embryonic plant tissue.

Rosen's (7) "belief" that "the continuous sucking action by the insect at one fixed point for fifteen days is the initial stimulus for gall development" will not hold, for there are too many non-gall-making hemipterous insects sucking at one place for extended periods without ensuing hypertrophy or hyperplasia. Further, as previously shown, little or no ingestion takes place, for in the early days of its gall-making activity no increase in size of the larva occurs. It is hardly conceivable that the pumping action of the insect's sucking apparatus would function without ingestion going on, especially when the proboscis end was in the presence of liquid or semi-liquid food. Attention may also be called to the fact that no experimental evidence along this line has yet been produced.

On *a priori* grounds it would seem much more natural to assume with Küster that such prosoplasmas were "*Chemomorphosen*"; that a highly specific chemical substance was introduced producing the radial effects noted. This conception, however, has not the slightest direct evidence for its support, since such a specific chemical substance producing a prosoplasma has never been demonstrated. The difficulty of demonstrating it may be of course due only to the exceedingly minute amounts of it which are formed. Conservatism here as elsewhere is the desirable position to assume.

One who has repeatedly removed these minute, delicate gall-inciting organisms from the plastic, watery tissue of the embryonic galls is impressed with the possibility of interpreting the situation in terms of correlation phenomena. The writer ventures to suggest that the insect may be acting as a whole in the matter.

Though the structural facts concerning gall initiation and early development are of much interest, we must confess that these data give us no more fundamental, explanatory information concerning gall ontogenesis than do the structural facts of normal growth explain normal ontogenesis. We must remain satisfied with having merely demonstrated the leading morphological facts involved in the early development of these two Pachypsylla galls.

Summary

1. The early stages of two hemipterous insect galls of the hackberry leaf were studied. The insects belong to the genus Pachypsylla of the Psyllidae.

2. The newly hatched nymphs after placing themselves upon the upper side of the young leaf initiate the following concomitant changes: (*a*) Formation by the cytoplasm of the affected cells of a sheath-like structure around the inserted setal proboscis. The cells are not killed. The pro-

boscis is inserted but once, the larva retaining its position through the period of gall development. (*b*) Hypertrophy of the epidermal and adjacent mesophyll cells on the lower side of the leaf opposite to that attacked by the insect. (*c*) A slight hyperplasia generally appears in the cells immediately surrounding the end of the mouth parts. (*d*) Degeneration of chloroplasts in a zone beneath the larva. (*e*) An increase in the size of the nuclei in the same zone.

3. The above noted changes (*b* and *c*) produce a downward evagination which lowers the insect into the body of the leaf, after which process a "cover-cone" rapidly grows over it, springing from the tissue surrounding the larva.

4. Multinucleate cells appear in the tissue of the floor of the larval chamber, most of the nuclei, however, soon disintegrating. The mode of origin of these nuclei is believed to be amitotic, though this under the condition of high divisional infrequency cannot be definitely proved.

5. The very early differentiation of a sclerenchyma layer, one cell in thickness, is highly characteristic of one of the galls.

6. The nymph shows practically no increase in size until the gall is about half developed. Thus the grand period of growth in the gall is at the beginning of its ontogeny while that of the insect is postponed until cecidiogenesis is well under way.

7. The morphological facts discovered furnish no assistance whatever in the formulation of a theory concerning the nature of the stimulus used by the larval insects in initiating and carrying to completion the highly specific gall structures characteristic of the two species respectively.

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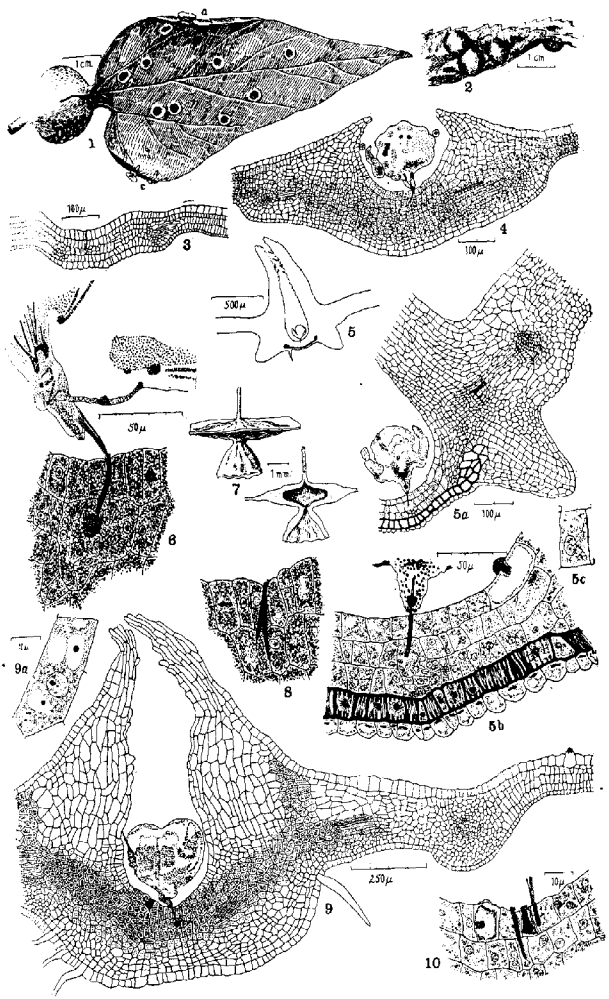

WELLS: EARLY STAGES IN THE DEVELOPMENT OF PACHYPSYLLA GALLS.

EXPLANATION OF PLATE XVIII

FIG. 1. Leaf of *Celtis mississippiensis* showing three distinct types of Pachypsylla galls. (*a*) Variety of *P. mamma*. (*b*) Compound gall of *P. venusta*. (*c*) Gall of *P. asteriscus*.

FIG. 2. Galls of *P. mamma* (typical form) on *Celtis occidentalis* leaf.

FIG. 3. Earliest initial stage of the *P. mamma* gall.

FIG. 4. Early stage of *P. mamma* gall.

FIG. 5. Outline of longitudinal section of early stage of gall of *P. asteriscus* (1 mm.).

FIG. 5*a*. Tissue study of lower right-hand portion of section shown in figure 5.

FIG. 5*b*. Cell study of region around mouth parts shown in figure 5*a*.

FIG. 5*c*. Multinucleate cell from same gall as figure 5 showing two nuclei, one of which has partially disintegrated.

FIG. 6. Detail from section of very young *P. mamma* gall; setae partially withdrawn from sheath.

FIG. 7. Gall of *P. asteriscus*, external and internal views.

FIG. 8. Detail from section of very young *P. mamma* gall showing the forked sheath formed through withdrawal and reinsertion of the setae.

FIG. 9. Section of very young *P. mamma* gall (1 mm.).

FIG. 9*a*. Cell from half grown *P. mamma* gall showing multinucleate condition. Note nuclei which show a degenerate condition.

FIG. 10. Detail from section of very young *P. asteriscus* gall showing three sheaths (more than one is extremely rare); setae still inserted in one to right.

THE UPWARD TRANSLOCATION OF FOODS IN WOODY PLANTS. II. IS THERE NORMALLY AN UPWARD TRANSFER OF STORAGE FOODS FROM THE ROOTS OR TRUNK TO THE GROWING SHOOTS?

OTIS F. CURTIS

There is apparently a very common belief that in most trees considerable quantities of the carbohydrates, that have been stored in the lower trunk and in the roots, move up as growth starts in the spring and are used in shoot and leaf formation.

The arguments which seem most commonly to be put forward as proof of such an upward transfer are that quantities of food are stored in the xylem tissues; that these are present in soluble form in the water-conducting vessels at the time spring growth commences; and that these foods rapidly disappear at about the time of this rapid shoot development. From these facts it would seem reasonable to think that the food had moved up with the water to the growing shoots, but, as shown in a recent paper (Curtis, 1920), the mere presence of soluble foods in water-conducting tissues cannot be considered as proof that the foods move with the water. In fact, it was shown that there is no appreciable longitudinal transfer of soluble foods through the xylem.

Some ringing experiments of Hartig's (1858) have also been considered as proof of the movement of foods from the roots to the growing shoots. At intervals of eight days from the first of April, 1857, until the middle of September of the same year, he ringed young oak trees of about the diameter of one's arm. The rings were two inches broad and were situated four feet from the ground. Some trees were also cut down at the time of ringing, but he does not state whether these were cut early or late in the season. Observations made in the spring of 1858 showed that all trees ringed previous to June 30, 1857, had lost the starch from below the rings, while those ringed after June 30 contained starch. The starch from these also, however, had disappeared by the fall of 1858. As the starch had not disappeared from some of the roots of the felled trees, he concluded that the food stored in the roots normally moves up with the water through the xylem and is used in shoot growth.

As has been previously shown, no appreciable quantities of food move longitudinally through the xylem and it seems very probable that the food below the rings disappeared because it was used in root growth and in diameter growth of the trunk. The only point tending to contradict this is that in some of the felled trees the starch did not disappear. This lack of

removal from the stumps of felled trees, however, may have been due to an excess of water following removal of the transpiring surface and resulting in a check on respiration or in death. When the stumps were healthy, as indicated by the development of shoots, the starch did disappear. Hartig failed to state the time at which trees were felled as well as the relative number of stumps which retained or lost their food stores.

The fact that the diameter growth of a trunk was very much decreased below a ring is additional proof that there is no large excess of food stored in the roots. Hartig explained this weak growth below a ring as due to the inability of the food, which he considered as moving up through the xylem, to move radially to the cambium. He believed that only that food coming through the phloem could be used in cambial growth.

Other data which have been considered as proof of the use of food from the roots for spring shoot growth have been presented by Leclerc du Sablon (1906) who determined the effects of ringing at different seasons on the amounts of carbohydrates found in roots and stems of a number of woody plants. He concluded that, as a general rule to which there may be exceptions, the roots of woody plants act as storage organs from which the carbohydrates move up in the spring. The data he offered, however, are far from conclusive. Some results he obtained in ringing experiments on the pear are presented in table 1.

The analyses for April 13 alone suggest that upward translocation from the roots might have taken place in the spring and that the ring has prevented this upward transfer, for in that tree ringed February 9 the roots

TABLE 1. *Data from Leclerc du Sablon to show effect of ringing on distribution of food between roots and stems of pear trees. Total carbohydrates expressed as percentage of dry weight.*

Date at which Sample Taken	Not Ringed		Ringed Feb. 9		Ringed May 8	
	Roots	Stems	Roots	Stems	Roots	Stems
Feb. 18	30.3	23.0	—	—	—	—
Apr. 13	22.4	21.3	25.6	18.3	—	—
June 16	27.9	23.7	27.9	29.5	17.5	29.0
Aug. 4	29.2	24.7	26.5	33.2	18.3	27.9
Sept. 24	33.8	25.7	19.3	29.1	21.4	29.5
Dec. 1	29.3	25.4	17.4	25.9	17.5	25.8

have a higher content than the check and the stems a lower content. But in a preliminary series (in 1904) he found similar differences between individuals taken at one time. For the quince, samples taken from four different plants on March 17 showed a maximum difference in carbohydrate content per 100 grams of dry material of 5.7 grams for roots and 4.9 grams for stems. With the pear, the corresponding differences were respectively 2.7 and 1.4. It is true that trees differing in external characteristics were definitely chosen for these samples, but similar differences might easily

have occurred between the other trees. Furthermore, the increases in carbohydrate content of the roots of ringed plants over that contained earlier in the season, shown on June 16 for those ringed on February 1 and as shown on June 16 and August 4 for those ringed May 8, would be hard to explain except as resulting from individual variations or from the healing of the wounds. In the quince, an analysis on April 13 showed greater carbohydrate content in the roots of the ringed tree than in those of the tree not ringed, but the stem of the ringed tree also showed a carbohydrate content greater than the stem of the check. Evidently the whole tree had a higher carbohydrate content.

Hartig, Leclerc du Sablon, Butler (1917), and others have shown that before growth starts in the spring the roots may contain a higher percentage of carbohydrates than the stems, but the stems have more supporting tissue and the percentage composition may therefore mean nothing unless the total mass is known. The actual amount of carbohydrates in the roots may be less than that in the tops, even though the percentage composition is high.

Data showing that the mass of carbohydrates stored in the roots is actually much less than that stored in the tops have been presented by Chandler (1917) who has calculated, from percentage concentrations obtained by Butler, the relative amounts of food available in the roots and tops of an apple tree. His data are presented in table 2.

TABLE 2. *Approximate amounts of dry matter, starch, and saccharose at the time buds are swelling, in case of a seven-year-old Bismarck apple tree that has been growing in sod.*

Part of Tree	Actual Dry Weights, Pounds	Pounds of Starch Calculated	Pounds of Saccharose Calculated
1-yr. twigs	3.15	0.98	0.12
Older branches	21.00	6.72	0.17
Trunk	15.13	5.14	0.11
Totals for parts above ground	39.28	12.84	0.40
Large roots	14.15	5.43	0.28
Small roots	6.49	2.37	0.06
Totals for roots	20.64	7.80	0.34

These figures are, of course, only suggestive, as the trees analyzed and the one weighed were grown under different conditions. But the error would tend to be in favor of large root storage, for the tree weighed had been grown in sod under conditions favorable to larger root growth. In this instance the roots weighed 52.5 percent as much as the tops. Pickering (1917) gives data showing the relative weights of tops and roots of a number of trees varying from 10 to 20 years old. The average root weight of 461 apple trees was 22.9 percent of the tops, that of 15 pears was 23.5 percent, that of 6 Damsons was 25.2 percent and that of 44 plums was 28.3 percent.

The relative root and top weights would, of course, vary with the soil and the climate, but there seem to be good indications that tree roots may not greatly exceed 50 percent of the top weight. Therefore, though the roots may have a carbohydrate content greater than the tops when measured as percentage of dry weight, the total quantity of carbohydrates in the roots is much less than that in the tops, and, since the roots must need quantities of food for their own use, it seems doubtful whether any is normally carried to the tops for shoot growth. The indications that root growth commences in the spring before shoot growth, as discussed later in this paper, may be considered as further proof that the food stored in the roots is used primarily by the roots.

Data obtained from experiments designed to determine the path of upward translocation, a subject reported in a recent paper by the writer (Curtis, 1920), offer evidence that little or none of the food stored in the trunks or roots of trees is normally moved up to be used by the developing shoots and leaves.

In one group of experiments, large numbers of twigs and branches were ringed early in the spring while the buds were still dormant or were just beginning growth. These rings were made at different distances from the tip in order to determine from how far back food was withdrawn for shoot growth.

Since, as was shown in the previous paper, no appreciable upward movement of foods occurs through the xylem, the growth of a shoot above a ring would serve as an approximate measure of the amount of food available. If the ring were back far enough from the growing tip to allow for growth practically as great as that on unringed twigs, it would seem that these twigs need not draw on the food stored at greater distances.

A large number of stems of *Acer saccharum* were ringed on April 5 at different distances from the tips. In one series the rings were in the first-year wood, in another in the second- or third-year wood, and in another the rings were in that part of the stem ranging from five to fifteen years old. Some of the stems had made terminal growths in the previous year of from only 1 to 10 centimeters, while others had made growths of from 20 to 40 centimeters. In each case a check stem was chosen as nearly matching the ringed one as possible. The check and ringed stems were usually the two terminals of a pair produced by dichotomous branching. Such a variety of branches was used that no attempt will be made to give more than a brief summary of the results.

Of 15 twigs ringed in the one-year-old wood, the average terminal growth on May 6 was 0.84 cm. That of the corresponding stems not ringed was 2.22 cm. Of those ringed in the two- and three-year-old wood the average terminal growth was 2.03 cm., while that of the corresponding checks was 2.25 cm. The leaves of the ringed stems in these cases did not show the bronze tinges that were common in the normal young leaves, but

were a bright green. At the same date, May 6, there were no apparent differences in the growths of stems not ringed and of those ringed below the 5- to 15-year-old stems. On May 25 measurements were made of these stems. An average of the shoots of ten stems of this series showed a growth of 9.96 cm., while that of the corresponding check stems was 10.09 cm. The older stems, whether the diameter was large or small, showed growth fully as great as that of those not ringed, but some of the younger stems showed somewhat lessened growth which lowered the average for the growth of ringed stems. In most cases growth had ceased and terminal buds were beginning to develop at the time of measuring.

On April 7, a number of stems of a pear tree growing in sod were ringed. These stems ringed in the one- and two-year-old wood showed distinctly lessened shoot growth, but those ringed where the diameter was from 1.5 to 3 cm. showed growth fully as great as that of the unringed stems.

On May 16, 1919, stems of an apple tree that was just beginning growth were ringed just below the base of the one-, two-, and five-year-old wood. At the time of ringing, the lengths of the shoots measured to the tips of the infolded leaves were from 1.5 to 2.0 centimeters. At the same time a single branch was ringed at its base where it measured 3.8 centimeters in diameter. The diameter of the main trunk just below the lower limbs was 11.0 centimeters. All the stems ringed in the fifth-year wood (group 4) were less than one centimeter in diameter at this point, with the exception of those lettered *c* and *j* which were respectively 1.2 and 1.5 centimeters in diameter. The growth was completed in most of the twigs when the measurements were taken on June 15. These data are recorded in table 3.

TABLE 3. *Pyrus malus. Ringed May 16, 1919. Measurements taken June 16. All of the same letter excepting in column 5 were closely matched. Column 5 and letters a to h on one tree, i to n on another. The data are represented as growth in centimeters.*

	1 Not Ringed	2 Ringed Below Base of One-year-old Wood	3 Ringed Below Base of Two-year-old Wood	4 Ringed Below Base of Five-year-old Wood	5 Ringed at Base of Branch 3–8 Cm. in Diameter
a.....	17.5	1.0	4.5	11.5	12.5
b.....	14.5	0.7	.5	5.0	13.0
c.....	9.5	0.4	3.0	10.0	13.0
d.....	15.5	0.4	2.0	9.0	12.5
e.....	17.0	*3.0 wound healed	6.0	21.0	10.5
f.....	16.0	1.0	—	11.5	
g.....	10.5	*5.5 wound healed	3.5	10.5	
h.....	10.0	2.5	*1.5 broken	—	
i.....	14.5	*2.5 wound healed	6.5	10.0	
j.....	19.5	0.4	4.5	13.5	
k.....	17.5	1.5	6.0	—	
l.....	18.5	1.8	7.0	—	
m.....	17.0	*6.5 wound healed	3.5	—	
n.....	14.5	*3.0 wound healed	4.0	9.5	
Ave.	15.14	1.08	4.42	11.15	12.3

* Not included in average.

From the table it seems that shoot growth is fairly vigorous when no food is further back than that obtained from a branch about one centimeter in diameter is available.

A somewhat similar experiment was tried with *Fagus grandifolia*. In this case the ringing was done before the buds had started. The data are reported in table 4.

TABLE 4. *Fagus grandifolia*. *April 7 to May 24.*

	Ave. Length of Shoot in Mm.	Ave. Number of Leaves
Twigs not ringed	186.4	6.6
Ringed in the middle of the one-year-old wood	21.4	2.1
Ringed at the base of the one-year-old wood	42.8	3.3
Ringed in the wood three to five years old which in all cases was less than one centimeter in diameter	59.1	5.0

These data as well as those reported in a previous paper (Curtis, 1920, tables 8–10) indicate that, when the ring is no further back than the 5- to 10-year-old wood, the growth of the shoots above the ring approaches more and more nearly that of the unringed stems. It will be necessary to use larger numbers of branches before one can attempt to state the distance from which food may be withdrawn.

Even if one could use large numbers of uniform stems that have been grown under uniform conditions, it would be difficult to determine from ringing experiments alone as to the exact distance of upward movement, for a check in growth may result not from lack of food but from lack of water due to the fact that no new xylem would be formed in the region of the ring, because in some trees much of the water may be carried through this new xylem. It is to be noted that, in practically every case in which the wound was not well protected by a coating of paraffin, the growth was distinctly decreased as a result of a deficiency of water due to drying of the xylem.

Not only is there considerable food stored in the twigs and young branches which becomes available for shoot growth in the spring, but the food manufactured by the new leaves soon after they open also becomes available for continued shoot growth.

The data reported in table 5 indicate that, soon after the shoots have started, much or all of the food necessary for continuing growth is produced by the leaves of that same shoot.

In the experiments with apple and in the first of the experiments with Ligustrum, the growth of the ringed twigs with leaves is fully as great as that of the twigs not ringed. In fact, in these two cases the data indicate that ringing has even increased growth above that in the checks. This may be because the ring has increased the food supply by preventing removal of that produced by the new leaves. In the other experiments, with the exception of the 1918 experiment with Philadelphus, the growth of the ringed

stems which retain their leaves is nearly as great as that of the normal stems. Even the results of the 1918 experiment with Philadelphus are not in opposition to the hypothesis that a large part of the food used for continued growth of a stem is produced by the leaves of that stem, for, as indicated in table 5 and also in the more detailed tables 1 and 2 of the previous paper, only about one third of the current year's growth was above the ring in the 1918 experiment and this part carried only the younger leaves, while in the 1919 experiment the entire new shoot with all its leaves was above the ring. The fact that the stems which were defoliated for a distance of from 15 to 20 centimeters from the tips in 1918 (group 3) showed such good

TABLE 5. *Experiment to determine how much food used in shoot growth may be produced by the leaves of that shoot.*

	1 Not Ringed, Leaves Remaining		2 Ringed, Leaves Remaining		3 Not Ringed, Leaves Removed		4 Ringed, Leaves Removed	
	Original Length of Shoot	Gain in Cm.	Original Length Above Ring, Cm.	Gain in Cm.	Original Length of Part Defoliated	Gain in Cm.	Original Length Above Ring	Gain in Cm.
Apple. June 11 to June 30. Ave. of 6 stems	25.0	4.48	15.0	5.3	15.0	3.37	15.0	0.25
Ligustrum ovalifolium. June 18 to July 3. Ave. of 7 stems		13.61	21.7	14.61	22.0	7.41	21.7	0.71
Ligustrum ovalifolium. June 19 to July 3. Ave. of 6 stems		12.41	20.8	10.58	20.9	5.75	21.1	0.23
Ligustrum ovalifolium. July 6 to July 22. Ave. of 6 stems		12.23	11.8	7.2	10.9	7.62	11.8	0.28
Ligustrum ovalifolium. July 11 to July 22. Ave. of 25 stems		9.94	17.8	8.19				
Philadelphus pubescens. May 30 to June 8, 1918. Rings in new growth. Ave. of 5 stems	54.6	34.1	17.0	11.75	17.0	27.0	17.0	0.5
Philadelphus pubescens. May 30 to June 4, 1919. Rings in old wood below base of new growth Ave. of 14 stems	18.8	16.36	21.1	12.96	17.4	7.96	18.5	1.16
Philadelphus pubescens. June 3 to June 6, 1919. Rings in old wood below base of new growth. Ave. of 8 stems	37.6	9.38	38.4	7.13	40.0	3.56	30.8	1.44

growth, while the shoots of the 1919 experiment (group 3) showed such poor growth gives additional proof that the leaves of the new shoot supply a large part of the food used in growth after a few leaves have once opened. In 1918 that food was available which was produced by the many leaves on the lower non-defoliated part of the stem, while in 1919 only stored food was available as the entire new shoot was defoliated.

Discussion

The available data are not sufficiently extensive to enable one to conclude from how far back food is withdrawn to be used in shoot growth. It is probable that the amount of upward movement depends upon the kind of tree, its age, and conditions of previous growth, as well as on conditions during the current season.

Leclerc du Sablon suggested that some trees may store but little of their food in the roots, while others store quantities there to be used later in shoot growth, but his experiments supposedly proving the latter condition are far from convincing. It is to be noted that he used young trees only three to four years old, and, though his data offer no conclusive proof, it is possible that such young trees might show more upward transfer of foods; but it is just as possible that, when a tree becomes well established, there is normally very little upward translocation. Other conditions being equal, one would expect little or no withdrawal of carbohydrates from below if during the spring growing season there were a deficiency of water and perhaps of mineral nutrients, especially nitrates, and the days were bright. Under such conditions vegetative growth would tend to become checked early, and the new leaves would soon begin to accumulate carbohydrates through photosynthesis.

If root growth commenced in the spring before shoot growth, or even if growth began in both at about the same time, one would expect that most of the food present in the roots would be immediately needed by the roots. No very conclusive evidence on this point is available, but Goff (1898) found that root growth may commonly precede the swelling of buds. Observations were made by digging trenches early in the spring and measuring the amount of new growth that had occurred up to the time of digging. Such early root growth was found to occur in the following plants: *Acer saccharum*, *Pyrus malus*, *Pyrus communis*, *Prunus cerasus*, *Prunus virginiana*, *Betula alba*, *Morus alba*, *Cornus stolonifera*, *Eleagnus hortensis* var. *Songorica*, *Ribes rubrum*, *Ribes nigrum*, *Ribes oxyacanthoides;* as well as in nine species of gymnosperms and a few herbaceous perennials. There were only two possible exceptions recorded.

Furthermore, data presented by Jones (1903) would indicate that root growth precedes stem growth. It was found that the water content of the trunk of the sugar maple increased from 31.5 percent in December to 36.5 percent in March, while from March 15 to April 28 the water content increased to 47 per cent. After this date the buds opened and the water content fell off. This rapid increase in water content just previous to the opening of the buds, which occurs not only in the maple but in all the other deciduous trees examined, though it cannot be considered as conclusive proof, yet at least suggests that the absorbing organs, the roots, have started growth early, making possible a rapid absorption of water just previous to shoot growth.

The data obtained from ringing dormant stems show that, when the ring is no further back from the growing tip than that part of the branch from 5 to 15 years old or from one to five centimeters in diameter, the growth may be practically as extensive as when no ring is made. When the growth was somewhat lessened by ringing, it may have been due, not to a lack of food, but to a deficiency of water, as the ring, of course, prevented the formation of a new layer of xylem. Furthermore, although the rate of starch loss was more rapid when the ring was near the tip, indicating more complete usage of stored food, yet the rate of starch loss when the ring was in the older wood was approximately the same as from a normal stem. These results indicate that normally very little food is withdrawn and carried up from the main trunk or roots to be used in shoot growth. It would seem therefore that, especially in older trees, the food in the branches is more than sufficient to initiate shoot growth, and that, since much of the food necessary for continued growth may be produced by the young leaves, there may be no tendency to draw upon that food stored in the roots. Furthermore, the fact that a cutting no longer than six inches may produce a short shoot with leaves and also a callus and roots, when no food can be obtained from storage organs outside that small bit of stem, would indicate that a shoot on a large branch need not draw on food stored at great distances, as from the trunk or roots.

There seems to be little foundation for the statement (Butler, 1917) that the carbohydrate stored in the young root tip is digested in the spring and carried up the trunk to the stems for shoot formation. It seems more probable that the roots themselves use nearly all, if not quite all, of the foods stored in them. When one considers that root growth probably commences earlier in the spring than shoot growth and may also continue later in the fall; that at no time can roots produce their own foods, as can the shoots as soon as a few leaves form; and that the tree roots probably store a smaller mass of food than do the tops, it is then difficult to see how food from the roots can be of very great aid in the shoot formation of a tree.

Summary

Facts commonly considered as proving that the food which is stored in the roots and lower trunks of many trees is carried up to be used in shoot formation cannot be considered as actually proving such upward movement.

When a ring is made on that part of a stem from 5 to 15 or more years old or from one to four or more centimeters in diameter, the growth above the ring approximates that of a normal stem, which fact indicates that upward movement of food from points below the ring is not essential.

When shoot growth is well started, much of the food used for continued growth may be produced by the leaves of that shoot, which fact indicates that considerable growth may take place when but little stored food is available.

The data at hand are not adequate to settle the question as to how far back from the tip food is withdrawn to be used in shoot growth. In fact, the amount of removal probably varies with different species and with different individuals, depending on the conditions of the current as well as of previous seasons. It seems very probable, however, that there is normally no upward movement of foods from the roots and perhaps little or none from the main trunk.

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EARLY STAGES IN THE DEVELOPMENT OF THE SPOROPHYTE OF SPHAGNUM SUBSECUNDUM

GEO. S. BRYAN

In the spring of 1913 while following through the life cycle of Sphagnum in the field the writer was able to find a great wealth of young sporophytic material. In order to make a detailed study several strengths and combinations of chromic and acetic acids were used as fixing agents. However, it was extremely disappointing to find that in every case the young sporophytes were completely plasmolyzed so that an interpretation of sectioned paraffin material was impossible. Time now being an important factor, as a last resort the dissection of the young sporophytes from the venters of the archegonia was undertaken. While the task was a laborious one, it proved to be relatively simple. The archegonia were first dissected from the tips of the branches on which they were growing. Then the basal portion of each venter, or, in the older stages, of the swollen stalk was carefully dissected, using slender needles for the operation. After the venter or the swollen stalk had been opened, gentle pressure on the neck of the archegonium was usually sufficient to cause the young sporophyte to slip out.

More than one hundred young sporophytes were examined. The study and drawings were made from the living material and are illustrative of the general conditions found. While the results confirm in a general way the work of Waldner, there are points of difference which make the publication of these observations seem worth while.

HISTORICAL

In 1858 Schimper[1] first attempted to trace the development of the sporophyte of Sphagnum. He described the first wall as being almost horizontal; then quickly follow radial, vertical, and horizontal walls, so that in a short time the single cell has become a long, many-celled, pear-shaped body. Schimper further thought that only the lower part of the young sporophyte—that which bores its way into the stalk of the archegonium—develops into the mature capsule. The upper portion he believed disintegrated and was resorbed.

In 1887 Waldner[2] studied the details of the development of the sporophyte using chiefly *Sphagnum acutifolium* Ehrh. He states that the egg

[1] Schimper, W. P. Versuch einer Entwickelungsgeschichte der Torfmoose. Pp. 96, pls. 1–27. Stuttgart, 1858.

[2] Waldner, M. Die Entwickelung der Sporogone von Andreaea und Sphagnum. Pp. 25, pls. 1–4. Leipzig, 1887.

in the venter of an archegonium ready for fertilization is ovoid or somewhat pear-shaped, and shows clearly a nucleolus with a nuclear body (this Taf. II, fig. 1). The fertilized egg is drawn very indefinitely, and no description is

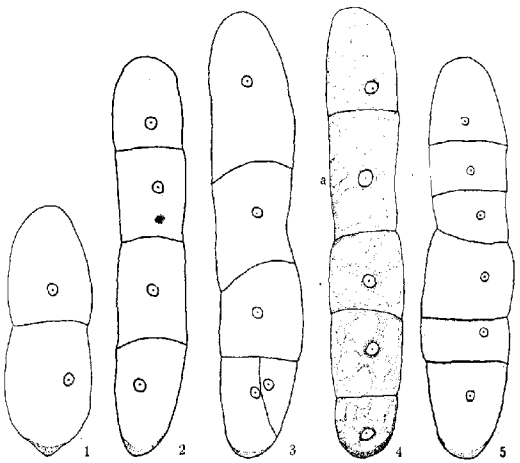

FIG. 1. Embryo of 2 cells. FIG. 2. Embryo of 4 cells. FIG. 3. Embryo of 4 primary segments. Apical cell much elongated, probably preparatory to division. Basal segment has divided irregularly. FIG. 4. Embryo of 5 cells, showing cytoplasmic details at this stage. FIG. 5. Embryo of 6 cells. All × 300.

given save that it is surrounded by a hyaline mass of slime (Taf. II, fig. 2). The fertilized egg divides transversely, the upper half being the apical cell. A two-celled embryo is drawn in which the upper cell is relatively small as compared with the basal segment. Waldner states that the apical cell, by walls parallel to its base, cuts off a series of segments from 6 to 8 in number. The basal cell makes only a few irregular divisions and does nothing more. Waldner's figures show that the wall separating the basal and apical segments is sharply defined and may be followed for some time in the subsequent stages of development of the young sporophyte.

FERTILIZATION, AND THE DEVELOPMENT OF THE YOUNG SPOROPHYTE

After a number of attempts, fertilization was brought about in the laboratory by squeezing the heads of antheridial plants and immediately allowing the exuding liquid to drop on the tips of archegonial plants which were almost entirely submerged in water. A detailed account of these experiments may be published at some later date. Unfortunately,

at the time of fertilization there occurs in the venter of Sphagnum the development of a considerable amount of mucilaginous matter which entirely invests the fertilized egg, and which thus far has proven difficult to penetrate

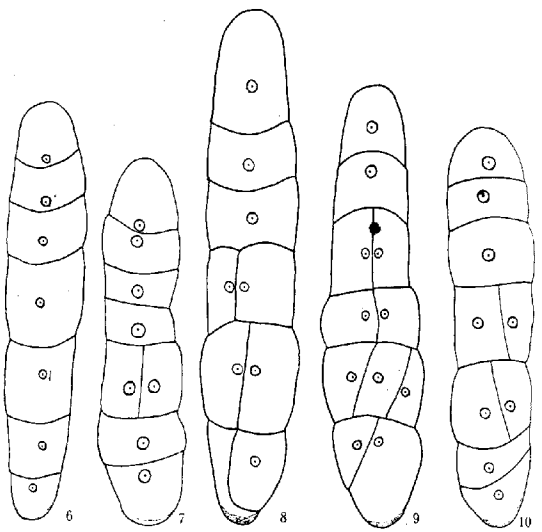

FIG. 6. Embryo of 7 cells. Slender type. FIG. 7. Embryo of 7 primary segments. First vertical wall appears in one segment. FIG. 8. Embryo of 6 primary segments three of which show vertical walls. The two cells of the basal segment are rounding away from each other. FIG. 9. Embryo of 6 primary segments, the basal portion much larger than the apical. FIG. 10. Embryo of 7 primary segments. All × 300.

with fixing agents. Since the results are not entirely satisfactory, because of plasmolysis, statements concerning the structure of the fertilized egg will be omitted.

The first wall is at right angles to the axis of the archegonium and divides the young sporophyte into two approximately equal cells which elongate in the direction of the axis of the archegonium (fig. 1). The basal cell is characterized at its lower extremity by a peculiar greenish zone which is probably related in some fashion to the digesting action of this cell. This zone is clearly visible in all the early stages (figs. 1–15) and serves as a convenient means of distinguishing the basal from the apical end of the embryo when dissected from the venter of the archegonium. After the two-celled stage has been reached there are further divisions by walls parallel to that first formed, resulting in the production of a filament of

cells usually six or seven in number before walls make their appearance in other planes (figs. 1–6). Occasionally, as illustrated by figure 3, the basal cell may divide quite early in other than a transverse plane. However, the filament of cells without such irregular divisions is the common occurrence in the material studied at these early stages.

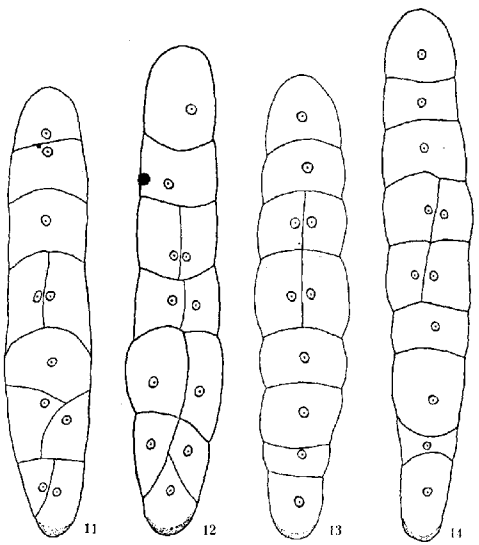

FIG. 11. Embryo of primary segments. Two basal segments show irregular divisions. FIG. 12. Embryo of 6 primary segments. Basal segment irregularly divided. FIGS. 13 and 14. Slender types. No irregular divisions in basal segments. All × 300.

In the later stages of development the writer has been unable to trace with any degree of certainty the original wall separating the basal and apical cells which Waldner in his drawings shows as sharply defined. As illustrated by figures 2–14, it is evident that in the material here studied there is no sharp delimitation, hence an exact statement as to the part contributed by each of these cells would be unwarranted. While no division figures could be found, the elongation of the apical cell as shown in figures 3, 8, and 12 furnishes strong evidence of apical growth, and finds frequent corroboration in the position of the nuclei as illustrated by the two uppermost cells in each of figures 7 and 11. Whether this apical growth may be supplemented by the occasional intercalary division of a primary segment is

questionable. Cell *a* in figure 4 suggests such a possibility. The elongation of this cell together with the size of the nucleus makes it seem probable that such a division is about to take place.

While it is impossible to trace with absolute certainty all of the divisions undergone by the basal cell, a study of basal regions shows two distinct types of divisions (figs. 2–21). As illustrated by figures 3, 8, 9, 11, and 12,

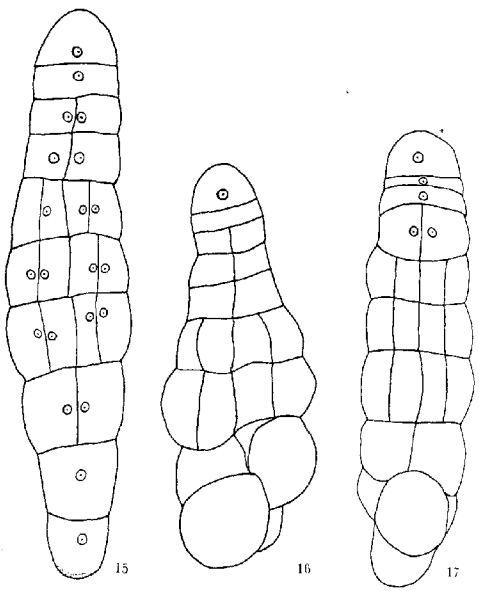

FIG. 15. Embryo showing 10 primary segments. Basal portion of sporophyte slender and without irregular divisions. FIG. 16. Bulbous type of young embryo. Cells of basal portion have strong tendency to round away from each other. FIG. 17. Embryo showing probably 9 primary segments. Basal portion bulbous. All × 300.

the basal cell divides irregularly, while in the embryos shown in figures 6, 7, 13, 14, and 15, if the basal cell has divided the divisions are regular, *i.e.*, brought about by walls parallel to that first formed. This irregularity or regularity expresses itself later in what might be termed two types of young sporophytes. The first of these, in which the lower portion is distinctly bulbous, is derived from the irregular type. The cells formed by these irregular divisions grow considerably in size and tend to round away from each other (figs. 16–17). Such embryos stand in sharp contrast to those of

the second type in which the basal cells are regular and slender and remain so for some time, apparently serving as a distinct boring organ aiding the young sporophyte in digesting its way into the stalk of the archegonium (fig. 15, 18, 20, 21). Such a structure is somewhat suggestive of an invaded suspensor. Figures 22 and 23, sketched from living material, give

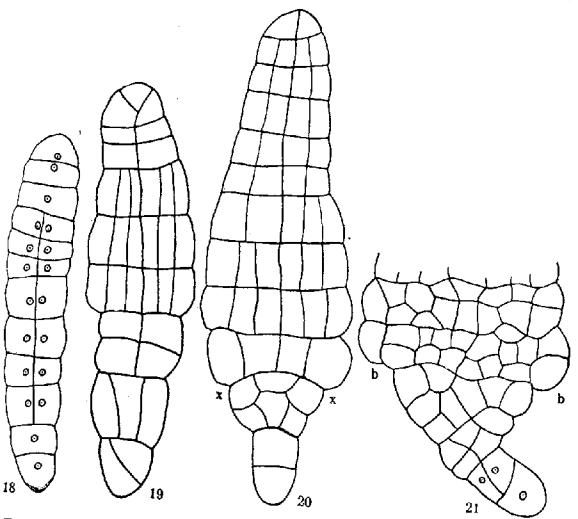

FIG. 18. Embryo showing 12 primary segments, the maximum number found in the material studied. The sporophyte is of the slender, regular type. FIGS. 19 and 20. Later stages of development. In the embryo shown in figure 20 the foot proper will develop above wall x–x. FIG. 21. Basal portion of young embryo at later stage than figure 20. The foot proper is beginning to develop along the region b–b. All × 180.

some idea of how quickly the young sporophyte bores its way out of the venter. In figure 22*a*, the embryo has already begun to digest the cells of of the venter immediately below it. In figure 22*b*, the embryo has worked its way entirely out of the venter and is embedded in the stalk of the archegonium. Figure 23 shows a still later stage in which the stalk of the archegonium has developed considerably in thickness, and illustrates the expansion of the basal portion of the young sporophyte to form the foot. It should be noted, however, that the immediate basal cells of the slender type do not form this expanded foot. In the embryo shown in figure 20, the foot will arise from the cells above the segment $x - x$. In figure 21 the expansion to form the foot proper can be observed at $b - b$.

An interesting situation is illustrated in figures 24–26. Wh[illegible] the dissections were first begun, a large percentage of the young sporo[illegible]ytes were found to be disintegrating. This disintegration, in all the [illegible]ases observed, begins with the apical cell and works downward. The [illegible]use

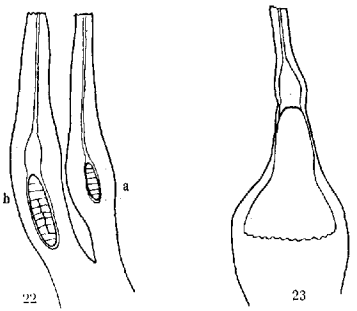

FIG. 22*a*. Young embryo beginning to digest the cells of the venter. FIG. 22*b*. The embryo has bored its way entirely out of the venter and lies embedded in the stalk of the archegonium. FIG. 23. Later stage showing the spreading of the foot. All × 60.

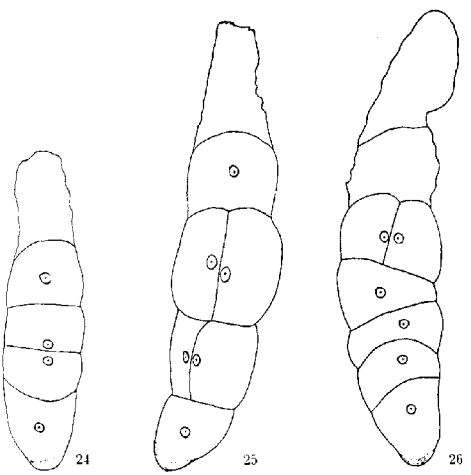

FIG. 24. Embryo of 5 cells. Apical cell has degenerated. FIG. 25. Older stage, apical cell degenerating. FIG. 26. Embryo of 7 primary segments, the two uppermost having degenerated. All × 300.

ld not be discovered. No fungus could be observed on or about the hegonia in these particular cases. The cells which had not disintegrated e apparently normal in every respect. It was unquestionably similar ervations which led Schimper to state that it is the lower part of the ing embryo which finally produces the sporophyte, the upper part ng and being resorbed.

SUMMARY

The main points of this paper may be summarized as follows:

1. The fertilized egg divides by a horizontal wall into two approximately equal cells. A filament of cells—6 or 7 in number—is usually formed before longitudinal divisions occur.

2. In the material studied the wall which separates the cells at the two-celled stage cannot be traced with certainty in the older stages, hence no exact statement can be made as to the contribution of each of these two cells in the development of the sporophyte.

3. It is reasonably certain that apical growth occurs.

4. The basal portion of the young sporophyte may have walls appearing in a regular or in an irregular order. As a result of the former process there is developed a long, slender type of young sporophyte; as a result of the latter a shorter, bulbous type.

5. The number of primary segments, *i.e.*, segments formed by walls transverse to the axis of the archegonium, has not been found to exceed twelve.

6. A considerable number of very young sporophytes show basipetal disintegration.

CONCLUSIONS

Much has been made of the striking character common to the Anthocerotales and the Sphagnales as contrasted with the remaining members of the Bryophyta—namely, the origin of the sporogenous tissue from the endothecium. The writer desires to point out the wide difference in early embryogeny. The general history in the Anthocerotales is the formation of an unequal quadrant, the two upper cells being somewhat larger than the basal ones. This stands in sharp contrast to the filament of six or seven cells produced in Sphagnum. An examination of the early embryogeny of the Bryophyta shows that the closest approach in this respect to the condition in Sphagnum is to be found among the Jungermanniales. Here a filament of three cells formed before the appearance of vertical walls is not uncommon.

If similarity in early embryogeny is significant in determining phylogeny, it must be evident that in this one respect Sphagnum shows a closer relationship to the Jungermanniales than to any other group of the Bryophyta.

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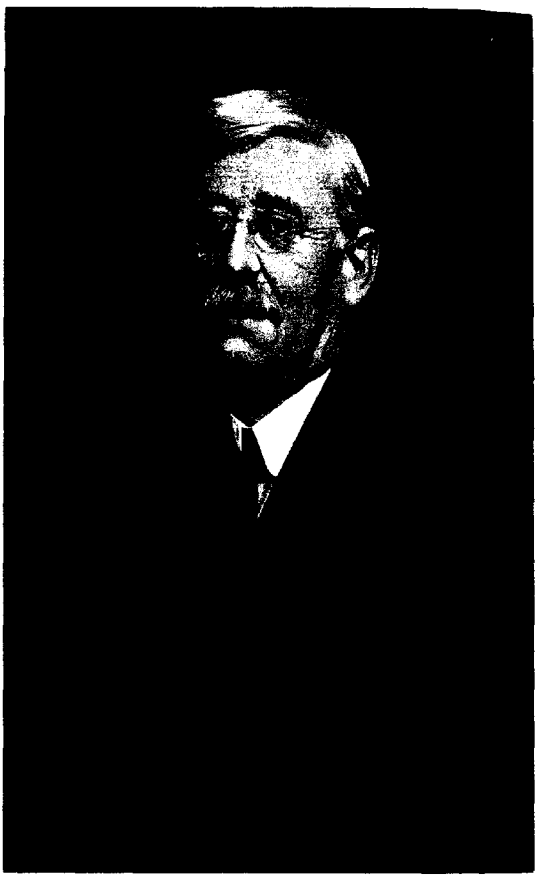

Yours sincerely
Byron D. Halsted